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PERKINS COIE LLP
P.O. BOX 2168
MENLO PARK, CA 94026

| EXAMINER | |
|---|---|
| FREDMAN, JEFFREY NORMAN | |

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**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Application Number: 09/867,201
Filing Date: May 29, 2001
Appellant(s): MAO ET AL.

LeAnn Gorthey
For Appellant

**EXAMINER'S ANSWER**

This is in response to the appeal brief filed May 24, 2007 appealing from the Office action mailed September 27, 2006.

**(1) Real Party in Interest**

A statement identifying by name the real party in interest is contained in the brief.

**(2) Related Appeals and Interferences**

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

**(3) Status of Claims**

The statement of the status of claims contained in the brief is correct.

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

**(4) Status of Amendments After Final**

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

**(5) Summary of Claimed Subject Matter**

The summary of claimed subject matter contained in the brief is correct.

**(6) Grounds of Rejection to be Reviewed on Appeal**

The appellant's statement of the grounds of rejection to be reviewed on appeal is substantially correct. The changes are as follows: In view of Appellant's amendment, cancelling claims 1, 2 and 4-8, the 102(b) rejection over Wong is no longer applicable to the claims. The 35 U.S.C 103(a) rejections remain applicable. So Issue 1 is necessarily overcome by the amendent, while Issues 2 and 3 remain.

**(7) Claims Appendix**

The copy of the appealed claims contained in the Appendix to the brief is correct.

**(8) Evidence Relied Upon**

| | | |
|---|---|---|
| 5,763,175 | BRENNER | 6-1998 |
| 5,935,793 | WONG | 8-1999 |
| 6,480,791 | STRATHMANN | 11-2002 |

For the above reasons, it is believed that the rejections should be sustained.

**(9) Grounds of Rejection**

The following ground(s) of rejection are applicable to the appealed claims:

Claims 1-12 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Brenner (U.S. Patent 5,763,175) in view of Wong (U.S. Patent 5,935,793).

Brenner teaches a method of sequence determination (see abstract) comprising:

(a) attaching an oligonucleotide tag from a repertoire of tags to each polynucleotide of the population to form tag-polynucleotide conjugates such that substantially every different polynucleotide has a different oligonucleotide tag attached (see column 2, lines 43-46, which teaches "An important aspect of my invention is the attachment of an oligonucleotide tag to each polynucleotide of a population such that substantially all different polynucleotides have different tags" and see column 20),

($b_1$) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment of the same size ladder having an end and the same oligonucleotide tag as every other polynucleotide fragment of the size ladder (see column 21, lines 29-37, where BbvI cleaves the tag-polynucleotide

conjugate to form size ladders)

($b_2$) wherein the generating step operates by shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides are shortened by a different amount (see column 21, lines 29-45, where the type IIs enzyme BbvI is used to shorten),

(b3) wherein extension products of known length are ligated onto each tag-polynucleotide (see column 21, lines 38-45, where S primers are ligated to the cleaved tag-conjugates).

(d) labeling the polynucleotide according to the identity of one or more nucleotides at the end of such polynucleotide fragment (see column 21, lines 20-25, where the polynucleotide is labeled),

(e) copying the labeled oligonucleotide tags of each polynucleotide fragment of each size class (see column 18, line 60 to column 19, line 31, where the isolated sequencing fragments are amplified by PCR, also see column 26, claim 1, step (e)),

(f) separately hybridizing the labeled oligonucleotide tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached as populations of substantially identical oligonucleotides in spatially discrete and addressable regions on one or more solid phase supports and the respective signature sequences being determined by the sequence of labels associated with each spatially discrete and addressable region of the one or more solid phase supports (see column 19, line 50 to column 20, line 46 and column 26, claim 1, steps (f) and (g)).

With regard to claims 2-3, 9, Brenner teaches where the type IIs enzyme BbvI is used to shorten the nucleic acids (see column 21, lines 29-45).

With regard to claims 4-5, Brenner teaches wherein extension products of known length are ligated onto each tag-polynucleotide (see column 21, lines 38-45, where S primers are ligated to the cleaved tag-conjugates).

With regard to claims 11-12, Brenner teaches extension oligonucleotides of about 12 nucleotides (see column 13, line 13).

With regard to claim 14, Brenner teaches the use of inosine (see column 20, lines 1-10).

Brenner does not expressly teach a step of generating a size ladder and separating the polynucleotide into size classes.

Wong teaches a method of claims 1 and 6 of sequence determination (see abstract) comprising:

(a) attaching an oligonucleotide tag from a repertoire of tags to each polynucleotide of the population to form tag-polynucleotide conjugates such that substantially every different polynucleotide has a different oligonucleotide tag attached (see column 7, lines 45-61, which teaches "'identifier tags', which are used to uniquely identify the sample fragment or template to which each tag is attached" and see column 25, claim 1, step b where tags are attached),

(b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment of the same size ladder having an end and the same oligonucleotide tag as every other polynucleotide fragment of the size ladder (see column 11, line 43 to column 12, line 64, where sequencing fragments

are generated, also see column 26, claim 1, substeps 3-5 of step (b)) (also related to the second interpretation in the claim interpretation section, see column 10, where Wong teaches cloning the polynucleotides into a vector to create multiple copies of the polynucleotide),

(c) separating the polynucleotide fragments into size classes (see column 17, line 20 to column 18, line 59 and see column 26, claim 1, step (c)),

(d) labeling the polynucleotide accoridng to the identity of one or more nucleotides at the end of such polynucleotide fragment (see column 19, lines 58-63, column 17, lines 10-35, column 14, lines 35-45, and column 27, claim 9, where "a different fluorescent label is used to identify each different terminating base type"),

(e) copying the labeled oligonucleotide tags of each polynucleotide fragment of each size class (see column 18, line 60 to column 19, line 31, where the isolated sequencing fragments are amplified by PCR, also see column 28, claim 10, step (d)),

(f) separately hybridizing the labeled oligonucleotide tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached as populations of substantially identical oligonucleotides in spatially discrete and addressable regions on one or more solid phase supports and the respective signature sequences being determined by the sequence of labels associated with each spatially discrete and addressable region of the one or more solid phase supports (see column 19, line 50 to column 20, line 46 and column 27, claim 10, steps (f) and (g)).

With regard to claim 4, Wong teaches forming extension products of known

length for each tag-polynucleotide (see column 11, line 43 to column 12, line 64, where sequencing fragments are generated).

With regard to claim 7, Wong teaches formation of a size ladder for each tag-polynucleotide (see column 17, line 20 to column 18, line 59 and see column 26, claim 1, step (c)).

With regard to claims 8, 10, Wong teaches physical separation using electrophoretic methods such as gel electrophoresis (see column 18, lines 1-37, for example).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify Brenner to incorporate the size separation as taught by Wong since Wong teaches "Conveniently, the sample or samples contain polynucleotide fragments within a selected size range, e.g., 400-2000 nucleotides, to achieve a desired sampling frequency for effective shotgun sequencing (see column 12, lines 6-16)."

The ordinary practitioner would have been motivated by Brenner to select a particular sampling frequency since Brenner wishes to avoid doubles and Brenner notes that "Thus, a design tradeoff exists between selected a large sample of target polynucleotides – which, for example, ensures adequate coverage of a target polynucleotide in a shotgun sequencing operation and selecting a small sample which ensures that a minimal number of doubles will be present (see column 11, lines 50-55)." An ordinary practitioner would therefore have been motivated to modify Brenner,

who is interested in selecting the correct amount of polynucleotide for shotgun sequencing, by using the size selection of Wong to achieve a sampling frequency expressly discussed by Wong as useful for shotgun sequencing.

Claims 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Brenner (U.S. Patent 5,763,175) in view of Wong (U.S. Patent 5,935,793) and further in view of Strathmann (U.S. Patent 6,480,791).

Brenner in view of Wong teach the limitations of claims 1-12 and 14 as discussed above.

Brenner in view of Wong do not teach the use of HPLC.

Wong does teach that "The size range may be controlled further by subjecting the sample to agarose, or polyacrylamide gel electrophoresis, size-exclusion chromatography, or other separation methods (see column 12, lines 12-15)."

Strathmann teaches that tagged polynucleotides can be separated by HPLC as a known equivalent chromatography method (see column 29, lines 25-64).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to utilize HPLC separation in the method of Brenner in view of Wong since Strathman teaches that HPLC is an known equivalent method which will function to separate the tagged nucleic acids by size and since MPEP 2144.06 notes " Substituting equivalents known for the same purpose. In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or

the mere fact that the components at issue are functional or mechanical equivalents. An express suggestion to substitute one equivalent component or process for another is not necessary to render such substitution obvious. In re Fout, 675 F.2d 297, 213 USPQ 532 (CCPA 1982)."

**(10) Response to Argument**

**Introduction**

This application is drawn to methods of determining nucleic acid sequences by tagging nucleic acids and forming size ladders of the nucleic acids, followed by the use of hybridization to detect which oligonucleotide was bound to which tag.

***Issue 1 - Do the teachings of Brenner in view Wong render the claims prima facie obvious?***

**Legal Standard**

The legal standard for obviousness is based upon the factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

As the Supreme Court recently noted, "Section 103 forbids issuance of a patent when 'the differences between the subject matter sought to be patented and the prior

art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.'" KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007).

**Prima Facie Case**

**Brenner teaches each limitation of the claims except using shortening to create a size ladder to form size classes and Wong teaches this element**

As the following analysis (and that shown in the rejection) demonstrate, Brenner teaches each and every limitation of the claims rejected under 35 U.S.C. 103 except using shortening to create a size ladder to form size classes.

| Claim 3 | Brenner |
|---|---|
| *A method of simultaneously determining a signature sequence for each polynucleotide in a population of polynucleotides, the method comprising the steps of:* | "A method for simultaneously determining the nucleotide sequences of a population of polynucleotides, the method comprising the steps of (see columne 27, claim 10, preamble)." |
| *(a) attaching an oligonucleotide tag from a repertoire of tags to each polynucleotide of the population to form tag-polynucleotide conjugates, such that substantially every different polynucleotide has a different oligonucleotide tag attached* | "An important aspect of my invention is the attachment of an oligonucleotide tag to each polynucleotide of a population such that substantially all different polynucleotides have different tags. (see column 2, lines 43- |

| | |
|---|---|
| | 46 and see column 27, claim 10, step a)." |
| *(b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated* | "The beads are then transferred to reaction mixtures containing Apa I, which cleaves all strands not containing methyl groups, i.e. all the strands that have been selectively amplified (see column 20, lines 60-63)." The PCR amplified products will inherently and necessarily be cleaved into different size polynucleotide fragments by ApaI since ApaI cleaves at every CCCGGG site which is not methylated. Since the PCR products, as noted by Brenner, are not methylated, ApaI will cleave at the ApaI site in the primer and any ApaI site naturally present in the cDNAs, which should randomly occur about every 4096 bases. |
| *(c) separating the polynucleotide fragments into size classes, wherein said steps of generating and separating include forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening* | "After, or concurrently with, the 32 PCRs, Bbv I is used to shorten the cDNA inserts of the library (see column 21, lines 39-41)." Also see "The amplicon is then cleaved with Bbv I and the S primer segment is removed |

| | |
|---|---|
| *by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount, and wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease* | with magnetic beads coated with avidin (see column 21, lines 42-45)". This step will generate polynucleotide fragments with the same tag with a Type IIs restriction enzyme. |
| *(d) labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of such polynucleotide fragment* | "labeling the copied tags according to the identity of the defined 3' terminal nucleotide of the second primer (see column 27, claim 10, step e and see column 21, lines 20-25, where the polynucleotide is labeled." |
| *(e) copying the labeled oligonucleotide tags of each polynucleotide fragment of each size class; an* | "copying the tags from the polynucleotides (see column 27, claim 10, step d and see column 18, line 60 to column 19, line 31, where the fragments are copied by PCR) |
| *(f) separately hybridizing the labeled oligonucleotide tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached as populations of substantially identical* | "sorting the labeled tags onto a spatially addressable array of tag complements for detection of the labeled tags and identification of the one or more nucleotides of each polynucleotide (see column 27, claim 10, step f) and see column 18, line 50 |

| | |
|---|---|
| *oligonucleotides in spatially discrete and addressable regions on one or more solid phase supports, and the respective signature sequences being determined by the sequence of labels associated with each spatially discrete and addressable region of the one or more solid phase supports* | to column 20, line 46)." |

While Brenner cleaves with ApaI, which will inherently form size classes, Brenner does not teach applying the use of the BbvI shortening enzyme to form the size classes and then separating the resultant polynucleotides into size classes.

Wong expressly teaches a method analogous to that of Brenner, in which size classes are formed. As Wong notes in claim 1 "c) separating said sequencing fragments on the basis of fragment length under conditions effective to resolve fragments differing in length by a single base, to produce a plurality of resolved size-separated fragments, (d) collecting the size-separated fragments in separate aliquots." Wong then amplifies (ie copies) the fragments and applies them to an array."

**<u>All feature are taught by Brenner with Wong</u>**

Appellant first argues that certain features of the invention are not taught by Brenner or Wong. Appellant states that Brenner does not teach generating a size ladder and forming size classes. In fact, as noted above, when Brenner cleaves with

ApaI, a size ladder will inherently be formed. Appellant is correct that Brenner does not expressly teach generating a size ladder in the shortening context with BbvI. However, the rejection is a 103 rejection in which Wong is relied upon for this element, not an anticipation rejection over Brenner alone. There is no doubt that Wong expressly teaches formation of size ladders, as Wong expressly notes, "The sequencing fragments are separated on the basis of size under conditions effective to resolve fragments differing in length by a single base. Such size separations may generally be accomplished by electrophoresis, chromatography, or other technique, provided that single base resolution is obtained. (see column 18, lines 10-15 of Wong)." Wong also teaches this concept in claim 1.

Appellant mischaracterizes Wong, when stating that because Wong does not teach shortening, the limitation of claims 2 and 3, Wong does not suggest size ladders. It is only this latter concept of size ladders for which Wong is required, not shortening. Brenner expressly teaches shortening with BbvI, noting "Bbv'I is used to shorten the cDNA inserts of the library (see column 21, lines 39-41)." Brenner simply does not teach using BbvI to shorten in the context of forming size ladders. Brenner forms a single size product.

The use of size ladders is prima facie obvious for several reasons when the teaching of Wong are included. First, there is express motivation to form ladders when Wong notes "Conveniently, the sample or samples contain polynucleotide fragments within a selected size range, e.g., 400-2000 nucleotides, to achieve a desired sampling frequency for effective shotgun sequencing (see column 12, lines 6-16)." Wong also

notes "If only one label type is used for detection, sequencing fragments may be processed together (i.e., separated by size .. (see column 17, lines 52-55)." These statements represent express motivation to select size ranges for analysis and this provides motivation from Wong to size select the polynucleotides in signature sequencing methods such as those of Brenner, in order to achieve a desired sampling frequency.

Separately, all of the claimed elements were known in the prior art and one skilled in the art could have combined the shortening with BbvI (expressly taught by Brenner) to form size ladders (expressly taught by Wong) with no change in the respective functions of the steps and the combination would have yielded the predictable result to one of ordinary skill in the art at the time of invention. In particular, cleavage of cDNAs without protecting the cDNAs by methylation would result in shortening of the cDNAs . When applied in the context of Brenner, the ordinary practitioner would have been able to shorten the cDNAs into different size classes to permit separation on electrophoresis gels as taught by Wong, in order to permit further differentiation of the target cDNAs as discussed by Wong with regard to the size of the fragments of interest (see column 12, lines 6-16).

**Claim 9 is suggested by Brenner and Wong**

Appellant argues that the limitation of claim 9 that a first primer is copied and then extened by ligation of an extension oligonucleotide is not taught by Brenner and Wong. In fact, Brenner expressly teaches,

> "After amplification, the amplicon is methylated to protect internal Bbv I sites, its 3' ends are stripped using T4 DNA polymerase and

> dGTP, after which the recessed strands are filled in by the addition of dTTP and dCTP. The amplicon is then cleaved with Bbv I and the S primer segment is removed with magnetic beads coated with avidin. The following adaptor mixture containing a new S primer binding site is then ligated to the T primer segment (see column 21, lines 38-45)."

This represents a precise teaching of the method required by claim 9. In claim 9, the primer must first be extended. As taught by Brenner, the primer is extended in an amplification reaction, as Brenner begins, "after amplification". The method is of the open "comprising" configuration and permits addiitional intervening steps before the ligation step. This initial amplification functions to copy the tag of the tag-polynucleotide conjugate into many millions of copies. Brenner then teaches that the cleaved adaptor is "ligated to the T primer segment", which can function as an "extension oligonucleotide".

When Appellant argues that Appellant's specification teaches that this will result in a size ladder, this argument fails both to combine Brenner with Wong and fails to address the claim as written. Claim 9 does not require that claim 9 steps actually form the size ladder. Appellant's entire argument is an attempt to read this limitation from the specification into the claims. In <u>In re American Academy of Science Tech Center,</u> 70 USPQ2d 1827, 1834 (Fed. Cir. 2004), the Federal Circuit noted "We have cautioned against reading limitations into a claim from the preferred embodiment described in the specification, even if it is the only embodiment described, absent clear disclaimer in the specification." The claim certainly lacks the requirement that this step form the size ladder.

Second, Brenner teaches the step, but does not teach the step in forming a size ladder. Wong teaches the desirability of forming size ladders as discussed above. Wong notes "Conveniently, the sample or samples contain polynucleotide fragments within a selected size range, e.g., 400-2000 nucleotides, to achieve a desired sampling frequency for effective shotgun sequencing (see column 12, lines 6-16)." This is an express motivation to select size ranges for analysis and this provides motivation from Wong to size select the polynucleotides in signature sequencing methods such as those of Brenner, in order to achieve a desired sampling frequency.

Appellant argues that this passage of Wong is drawn to sequencing of end regions. This passage refers to a desire to have different size targets in the analysis. Wong then provides a variety of different size targets expressly in the Wong method, Wong also notes "If only one label type is used for detection, sequencing fragments may be processed together (i.e., separated by size .. (see column 17, lines 52-55)." There can be no more direct statement by Wong that different size fragments can be used in the analysis method.

**Claim 13 is suggested by Brenner, Wong and Strathmann**

Appellant relies upon overcoming the rejections of Brenner in view of Wong to overcome this rejection. Since the rejection of Brenner in view of Wong should be sustained for the reasons given, this further rejection should also be sustained.

Respectfully submitted,

JEFFREY FREDMAN
PRIMARY EXAMINER
7/27/07

Conferees:

Robert Wax, TQAS Appeals Specialist

ROBERT A. WAX
PRIMARY EXAMINER

Gary Benzion, SPE, Art Unit 1637

GARY BENZION, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600